DEVELOPMENT OF IONTOPHORETIC DELIVERY SYSTEM

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

Bachelor of Technology
(Biotechnology)

Submitted
By

PRASANJEET PATTNAIK
Roll No. 107BT004

Under the Guidance of
Dr. Kunal Pal

Department of Biotechnology & Medical Engineering
National Institute of Technology
Rourkela 769008
2011

DEPARTMENT OF BIOTECHNOLOGY & MEDICAL ENGINEERING,

NATIONAL INSTITUTE OF TECHNOLOGY-ROURKELA

Dated: May 15, 2011

CERTIFICATE

This is to certify that the thesis entitled "**DEVELOPMENT OF IONTOPHORETIC DELIVERY SYSTEM**" submitted by **Mr. PRASANJEET PATTNAIK** in partial fulfillment for the requirements for the award of Bachelor of Technology Degree in Biotechnology at National Institute of Technology, Rourkela is an authentic work carried out by him under the supervision of the undersigned.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

(Dr. KUNAL PAL)
Assistant Professor

ACKNOWLEDGEMENTS

I owe a great many thanks to great many people who helped and supported me for the completion of this project effectively and moreover in time.

My deepest and sincere thanks to Dr. Kunal Pal**,** Assistant Professor, Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela for giving me an opportunity to carry out this project under his supervision. He has been very kind and patient while suggesting the outlines of the project and has also been very helpful in the successful completion of the same. I thank him for his overall support.

I am equally thankful to the Ph.D scholars, Mr. Sateesh Sagiri and Ms. Beauty Behera, Department of Biotech & Medical Engineering, National Institute of Technology Rourkela for their support and guidance.

Finally, let me say "Thank You" to my friends Mr. Sarada Prasanna Mallick and Mr. Ankurman Shrestha for their encouraging words and motivation.

Lastly I express my abysmal adoration and heartfelt devotion to my beloved parents for their countless blessings, unmatchable love, affection and incessant inspiration that has given me strength to fight all odds and has shaped my life and career till today.

In the end I must record my special appreciation to the Almighty who has always been source of my strength, inspiration and my achievements.

Date: 15-05-2011 ***Prasanjeet Pattnaik***

CONTENTS

Abstract

The current study deals with the development of a low-cost iontophoretic delivery system. Iontophoresis deals with the administration of the drug under the influence of electrical potential. A low-cost portable and user friendly pulsed iontophoretic system was developed. The efficiency of the system, at various duty cycles, was analysed against the salicylic acid (SA)-loaded gelatin gels. The current density for the studies was maintained at 0.5 mA/cm^2. The results indicated that as the duty cycle was increased there was a subsequent increase in the drug release from the gelatin gels. 100 % duty cycle provided the best release behaviour in terms of % SA release. Taking this as the reference duty cycle, the SA release from the alginate-gelatin and chitosan-gelatin gels were also studied. The results indicated that as the proportion of alginate was increased in the alginate-gelatin gels, there was an increase in the % release whereas there was a decrease in the SA release as the chitosan proportion was increased in the chitosan-gelatin gels. The release patterns indicated that the release from the gels followed Higuchian kinetics.

Keywords: Iontophoresis, Electric Potential, Current density, Higuchian kinetics

List of Figures **Page No.**

List of Tables **Page No**.

Chapter 1

INTRODUCTION

1.1. Background

Iontophoresis may be defined as a phenomenon, which involves the use of electric potential to drive an ionic solute into the systemic circulation without any invasive procedures [1]. This technique has been classified as transdermal delivery system [2]. The non-invasive nature of the method has led to the increase in the patient compliance [3]. This has been attributed to the various disadvantages of the conventional delivery systems, *viz.* oral and parenteral delivery systems. Since the procedure allows the administration of the drug directly into the systemic circulation, various drugs with short shelf-life may also be tried as a formulation [4]. The potential of this technique has been exploited by various researches for the transdermal delivery of many drugs with poor penetration properties (e.g. high molecular weight electrolytes such as proteins, peptides and oligonucleotides) which are normally difficult to administer except through parenteral route [5]. Most of the drug molecules are ionic species which allows the administration of the drug using electric potential by facilitating its easy penetration through the skin into the systemic circulation [6].

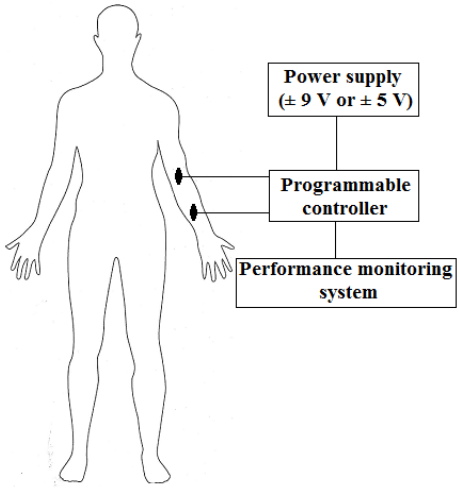


Figure1. Schematic diagram of an iontophoretic system

The schematic diagram of an iontophoretic system has been shown in figure 1. It is clear from the diagram that an electric current has to pass through the patient's body for delivering the drugs. This is because a stringent electrical safety protocol has to be followed. As a matter of fact, various approaches have been used for ensuring patient safety. The methods which have gained much importance include the use of a battery powered system and the use of dc-dc converters (figure 2). Apart from the above, US FDA has set the maximum limit for the current density through the electrodes as $0.5 mA/cm^2$ for a typical iontophoretic system to avoid any discomfort to the patients [7].

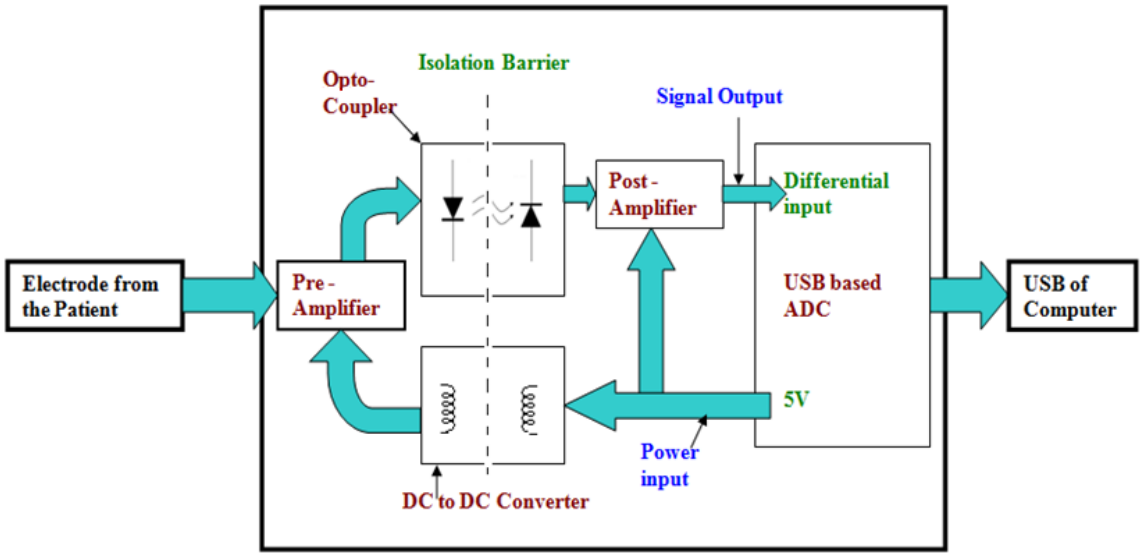


Figure2. Schematic diagram of a power isolation circuit employing dc-dc converter for patient isolation

1.2. Project Outline

The current study deals with the development of a low-cost portable iontophoretic system. The efficiency of the system was determined against SA-loaded gelatin gels. Attempts were also made to study the effect of polyelectrolytes (e.g. sodium alginate and chitosan) on the release pattern of the SA.

Chapter 2

Materials and Methods

2.1. Materials used

Atmega-32 MCU was procured from Atmel, CA, USA while JHD 162A LCD module was procured from Hitachi India, New Delhi, India. A 4x4 matrix keypad was obtained from Robokits, Ahmedabad, India. All other electronic components (e.g. D880 power transistor, 470Ω preset, transistor BC 548, LM 317) were procured from the local market.

Tween 80 (polyxyethylene sorbitan monooleate) and gelatin (commercial grade) were procured from Himedia, Mumbai, India. Ethanol was obtained from Honyon International Inc., Hong Yang Chemical Corpn., China. Glutaraldehyde (25%, for synthesis; GA) and hydrocholric acid (35%pure) was obtained from Merck Specialities Private Limited Mumbai, India. Salicylic acid (SA) were procured from Loba Chemie, Mumbai, India

2.2. Circuit designing

The iontophoretic driver circuit should be able to deliver constant current for a particular experimental condition. LM 317 (variable voltage regulator, to be in making a constant current source) was used for maintaining constant current during an experiment. It is desirable to include suitable LED indicators which may give indications about the running modes. The circuit was developed as per the figure 3 and was regarded as driver circuit.

Atmega-32 (8-bit) microcontroller unit (MCU) was used to drive the driver circuit at various duty cycles (e.g. 0 %, 20 %, 40 %, 50 %, 60 %, 80 % and 100 %). The microcontroller module was then augmented with a LCD module and a matrix keypad, so as to bring-in easier user interface. The schematic diagram of the whole system has been given in figure 4 (real picture can be found in Appendix 1 and MCU code can be found in Appendix 2).

Patient isolation modules can also be augmented with the power source so as to ensure patient's safety when the device is ready to be used on humans.

2.3. Circuit diagram

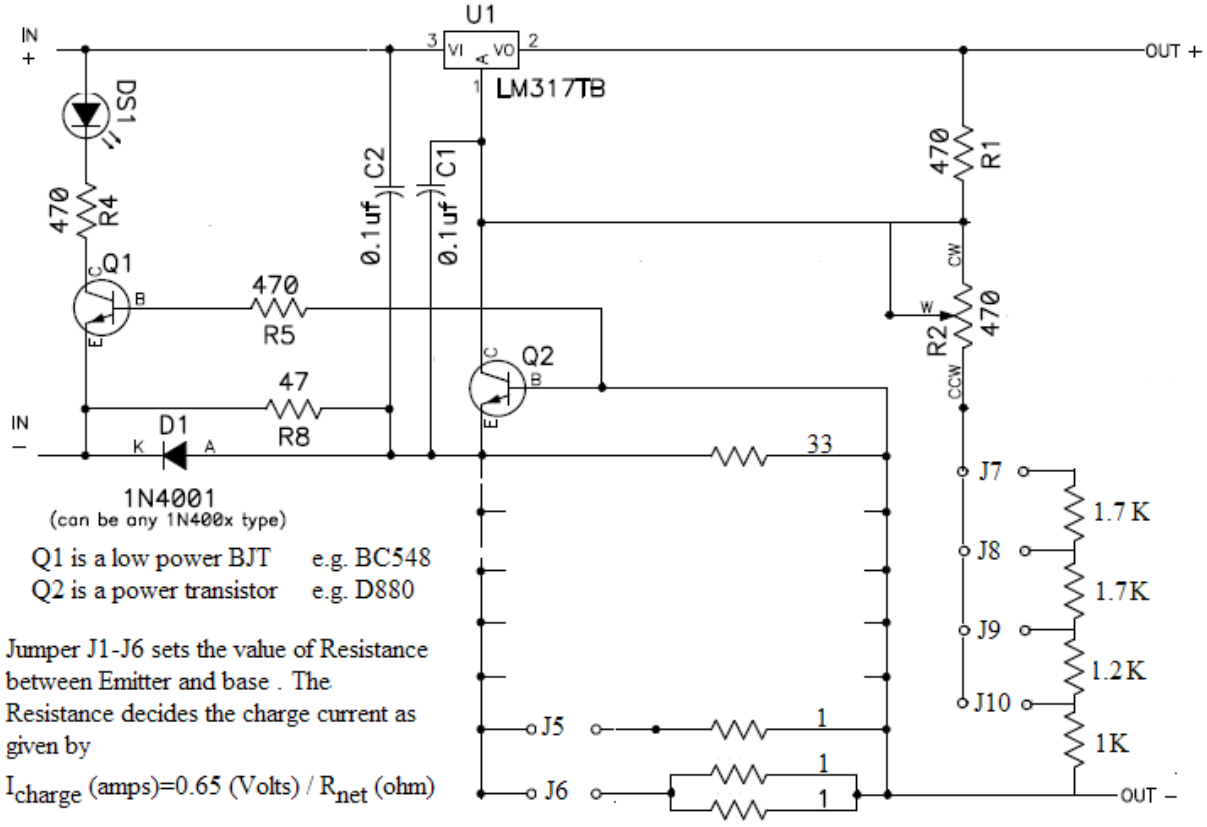


Figure 3.Circuit diagram sowing all the essential components

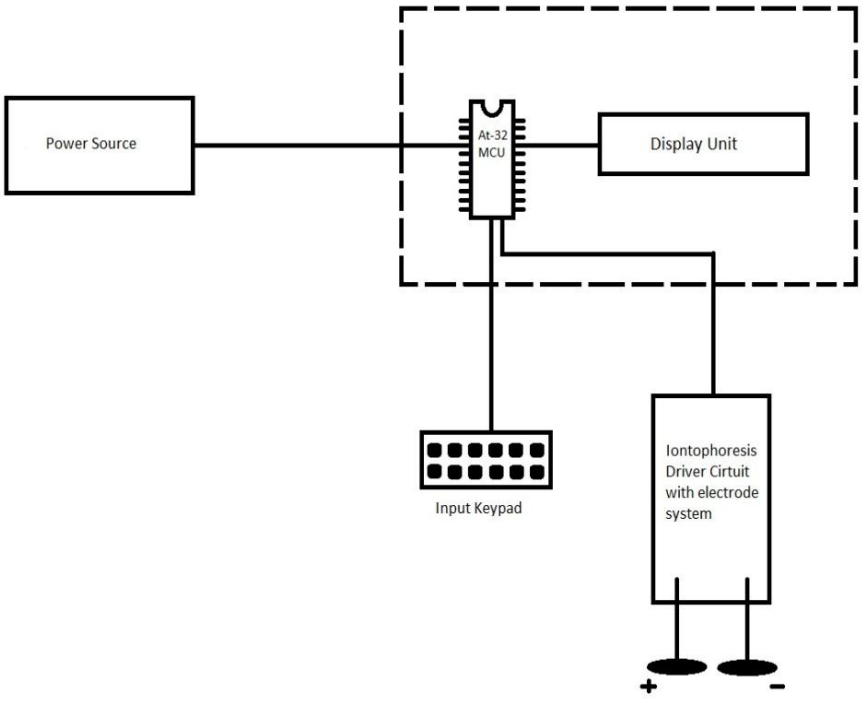


Figure 4.Schematic diagram of the entire iontophoresis assembly

2.4. In vitro drug release

In vitro drug release was conducted in a specially designed diffusion cell (figure 6). The diffusion cell consists of two donor compartments. The receptor compartment consisted of two ports, which allowed the attachment of the donor compartments through semi-permeable membranes. Apart from this, receptor compartment also contained a sampling port for sampling of the receptor fluid. A schematic diagram of the diffusion cell has been shown in figure 5.

The matrix for the donor compartment with active electrode (cathode) contained SA-loaded gelatin gels. The SA-loaded gelatin gels were prepared by mixing 4 ml of 10 % (w/v) SA solution with 16 ml of 10 % (w/v) warm gelatin solution [8]. This was followed by the addition of the glutaraldehyde reagent (0.5 ml GA + 0.5 ml ethanol + 0.1 ml of HCl). The solutions were mixed thoroughly for 30 sec and immediately poured into the donor compartment for inducing gel formation. The remaining space, if any, was filled with blank gelatin solution. In the similar manner the passive electrode (anode) was also filled with gelatin-only gels (16 ml gelatin sol) containing 4ml of normal saline. Thereafter, the donor compartments were brought in contact with the receptor compartments, filled with distilled water and having cellulose acetate membrane as the semipermeable membranes and pulsed potential of various duty cycles were applied. Care was taken so that the current density did not increase 0.5mA/cm^2. At regular intervals of time, 10 min for first 1 h and 20 min interval for the next 2 h, samples of 3 ml was drawn from the receptor and was replenished with fresh 3 ml water. The sampled solutions were analysed using UV-vis. spectrophotometer (Sistronics Double Beam Spectrophotometer model number 2303) at 294 nm.

In similar experiments, various proportions of chitosan-gelatin and alginate-gelatin blends were used to make the donor matrices (table 1a & 1b). 2.5% of chitosan solution was prepared by dissolving 0.5 g of chitosan in 8 ml of 99.5% glacial acetic acetic acid and then taking finally diluting with 12 ml of water. 10% of alginate solution was prepared by dissolving 2 g of alginate in 20 ml of water. The polyelectrolyte and gelatin solutions were mixed in various proportions and treated with GA reagent to develop matrices as describe in the previous paragraph. The duty cycle, which provided best result with the gelatin gels, was used in the study to compare the effects of the polyelectrolytes on the drug release properties.

Table 1a: Table of Compositions for Gelatin-Chitosan gels

Sample	Vol. of Gelatin sol (ml)	Vol. of Chitosan sol (ml)
GC0	20	0
GC1	12	8
GC2	8	12
GC3	0	20

Table 1b: Table of Compositions for Gelatin-Alginate gels

Sample	Vol. of Gelatin sol (ml)	Vol. of Alginate sol (ml)
GA0	20	0
GA1	12	8
GA2	8	12
GA3	0	20

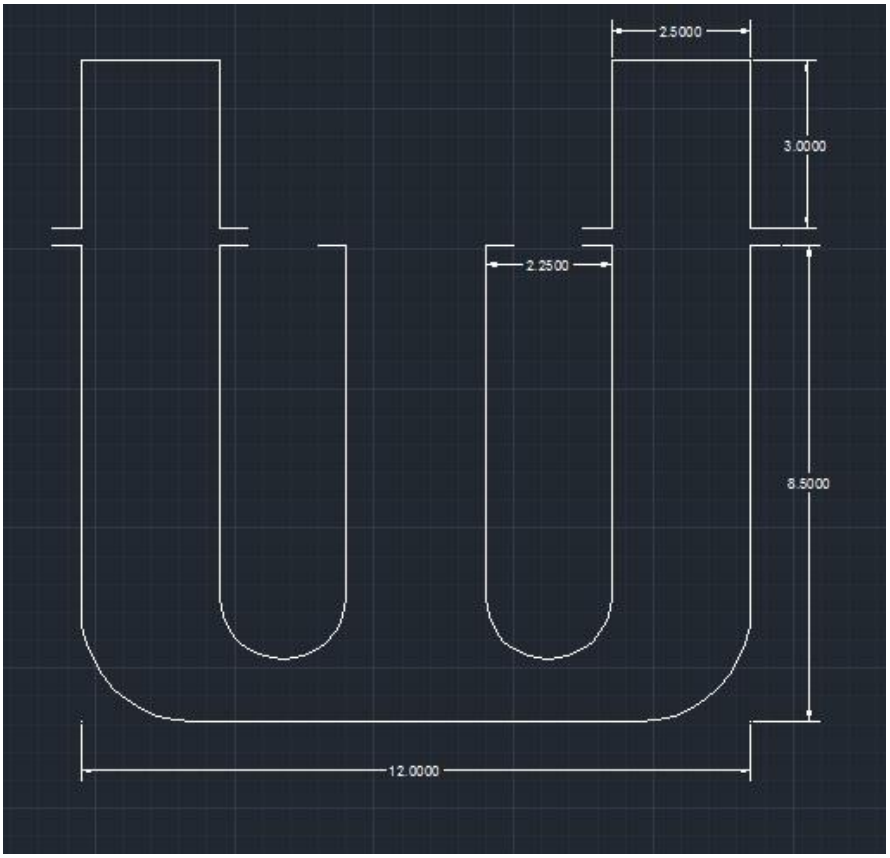


Figure 5.CAD model of the diffusion apparatus (all dimensions in cm)

Chapter 3

RESULTS and DISCUSSION

3. Drug release study of Gelatin gels

The preliminary experimentations were carried out using SA-loaded gelatin gels as the conducting matrices. They were subjected to different duty cycles of pulsed dc (Vp= 10.5) voltages. The release profile of SA through the gelatine gels have been shown in figure 6. The results indicated that as the duty cycle was increased from 0% to 100 %, there was an increase in the rate of release of the drug. The % release of the drug varied from 67.1 % during passive diffusion and 72.22 % when 100 % duty cycle of the dc voltage was applied.

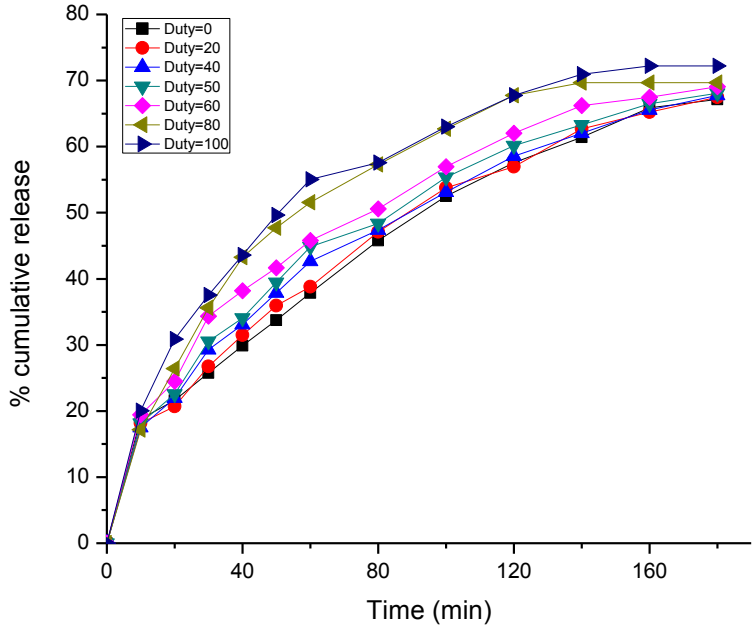


Figure 6.Graphical plot of percentage drug released vs. time, Gelatin gels

The release kinetics of SA from the gelatin gels were studied by analyzing for zero order, first order and Higuchian models. The results of the kinetics studies have been tabulated in table 2. The results indicated that the release of SA from the gels followed Higuchian kinetics [9], (table 2 and figure 7). Since the SA-loaded gelatin gels contained SA molecules uniformly distributed throughout the gelatin matrices, this can explain the Higuchian release kinetics for SA from the geltain gels.

Table 2: R^2values for Gelatin gels

Duty cycle (%)	r^2 for model fitting			Best fit
	Zero order model	First order model	Higuchi model	
0	0.88102	0.92398	0.96899	Higuchi
20	0.9066	0.94532	0.97967	Higuchi
40	0.9285	0.96494	0.99153	Higuchi
50	0.93258	0.9685	0.98513	Higuchi
60	0.90575	0.95065	0.98396	Higuchi
80	0.94534	0.98172	0.98614	Higuchi
100	0.9324	0.97955	0.98704	Higuchi

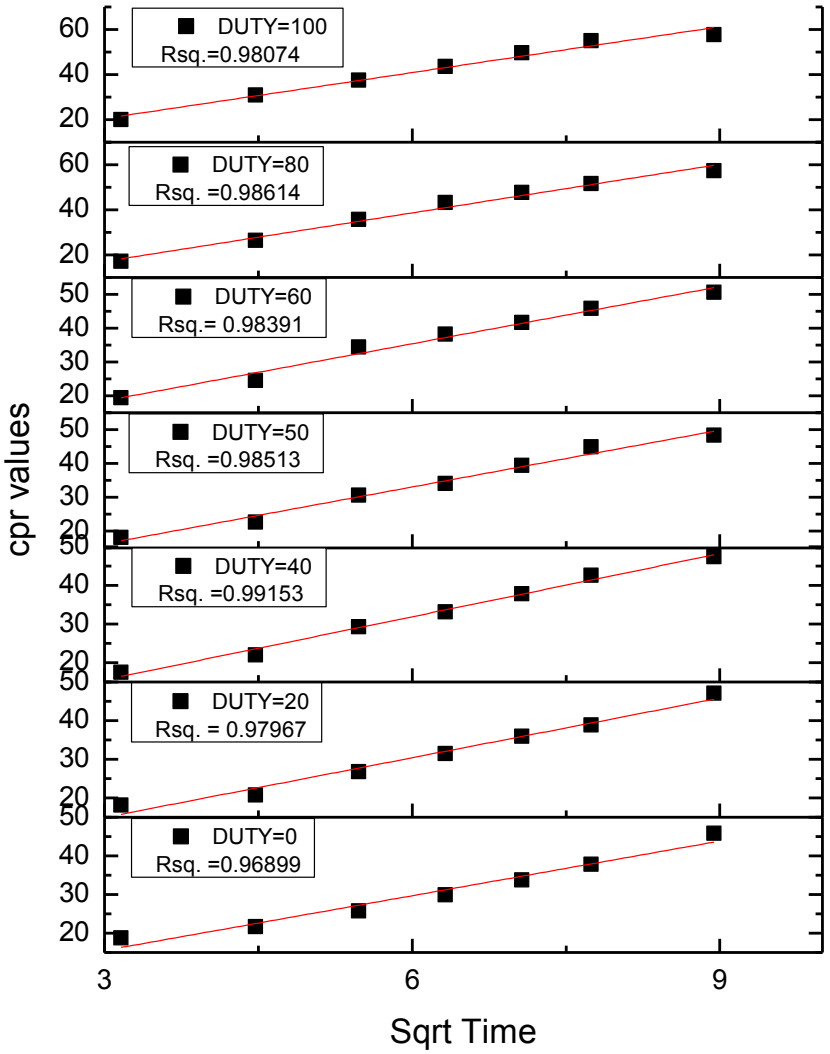


Figure 7.Graphical plot of percentage drug released vs. sqrt. time (Higuchi model), Gelatin gels

Gelatin is a neutral polymer. To determine the effect of the polyeletrolytes (e.g. chitosan and alginate), another set of experiment was carried out by preparing matrices by blending chitosan & gelatin and alginate & gelatin polymers. Since 100 % duty cycle gave us the best release profile from the SA loaded gelatin gels, it was selected for the further studies with the polyelectrolyte matrices. When chitosan and alginate was used as polyelectrolytes, the pulsed dc with Vp of 8.2 was used. This was done to ensure that the current density did not increase beyond 0.5 mA/ cm^2. When chitosan was used as polyelectrolyte, the release results indicated that with the increased proportion of chitosan in the matrix, there was a marked decrease of the SA release (figure 8). For GC3, the release was found to be 34.66 % whereas when GC0 was used, the release was found to be 63.00 %. When alginate was used as polyelectrolyte, the release results indicated that with the increased proportion of alginate in the matrix, there was a marked increase of the SA release (figure 9). For GA3, the release was found to be 71.59 % whereas when GA0 was used, the release was found to be 63 %.

The observed release results may be explained by the fact that the SA, being an anion, interacts ionically with the cationic amino groups of the chitosan polymer. This plays an important role in hindering the release of SA from matrices. With the increased amount of chitosan, the interaction amongst the SA and the chitosan becomes stronger and hence the lower % release of SA (figure 8). On the other hand, SA and alginate, both being anions, repel each other with minimal associative interactions amongst them. Hence, as the potential is applied, it provides an additional outward thrust on the SA molecules thereby resulting in the quick release of the SA molecules from the matrices. With the increased amount of alginate, the interaction amongst the SA and the matrices becomes weaker and hence the higher % release of SA (figure 9). Gelatin being a neutral polymer, showed release profile in between the chitosan and alninate matrices (figure 8 and 9).

The release kinetics of SA from the gels was studied by analyzing for zero order, first order and Higuchian models. The results of the kinetics studies have been tabulated in table 3a and 3b. The results indicated that the release of SA from the gels followed Higuchian kinetics (table 3a, 3b and figure 10, 11).

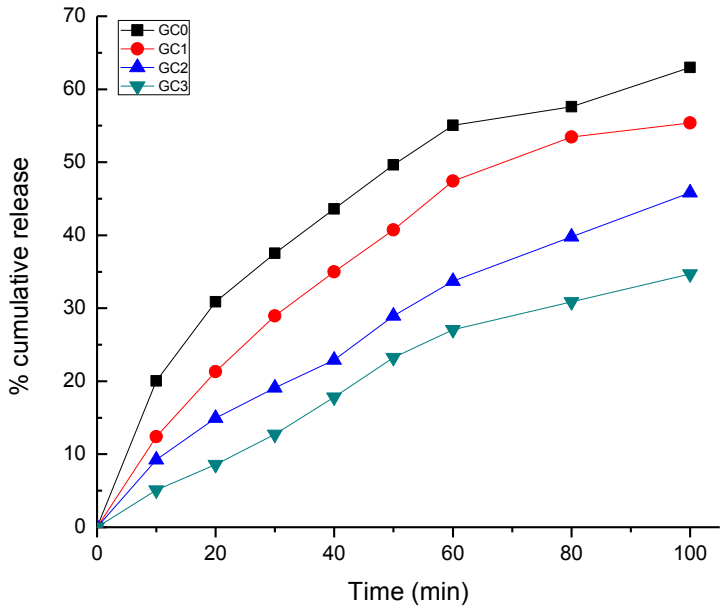


Figure 8.Graphical plot of percentage drug released vs. time, Gelatin-Chitosan gels

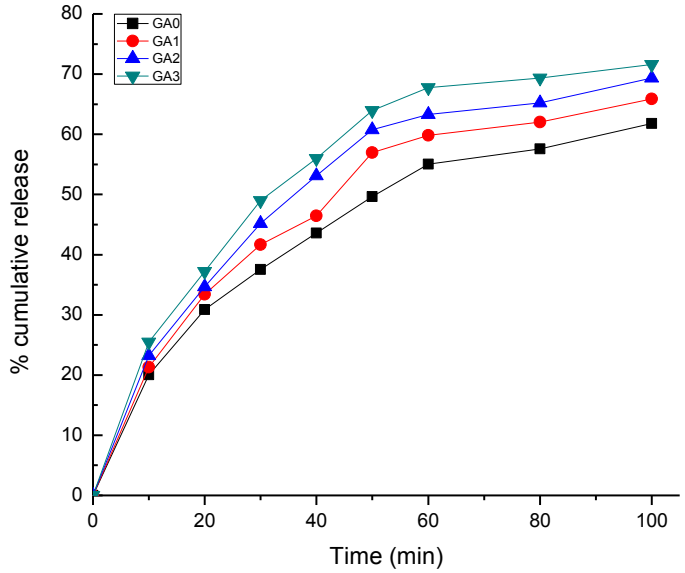


Figure 9.Graphical plot of percentage drug released vs. time, Gelatin-Alginate gels

Table 3a: R^2values for Gelatin-Chitosan gels

Sample	r^2 for model fitting			Best fit
	Zero order	First order	Higuchi model	
GC3	0.96591	0.97949	0.98339	Higuchi
GC2	0.97358	0.99335	0.9943	Higuchi
GC1	0.91704	0.96272	0.98174	Higuchi
GC0	0.85559	0.93902	0.97609	Higuchi

Table 3b: R^2values for Gelatin-Alginate gels

Sample	r^2 for model fitting			Best fit
	Zero order	First order	Higuchi model	
GA3	0.78491	0.8826	0.91781	Higuchi
GA2	0.80404	0.9016	0.93069	Higuchi
GA1	0.82473	0.9118	0.94564	Higuchi
GA0	0.85559	0.93902	0.97609	Higuchi

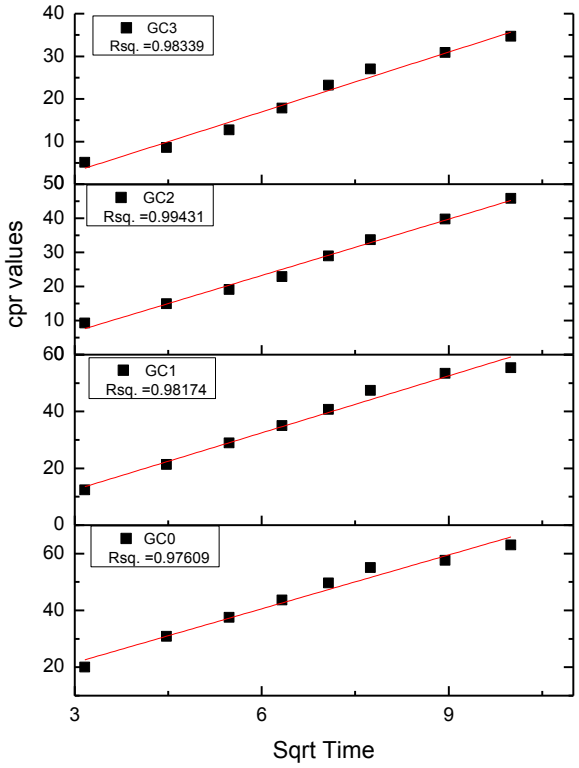


Figure 10.Graphical plot of percentage drug released vs. sqrt. time (Higuchi model), Gelatin-Chitosan gels

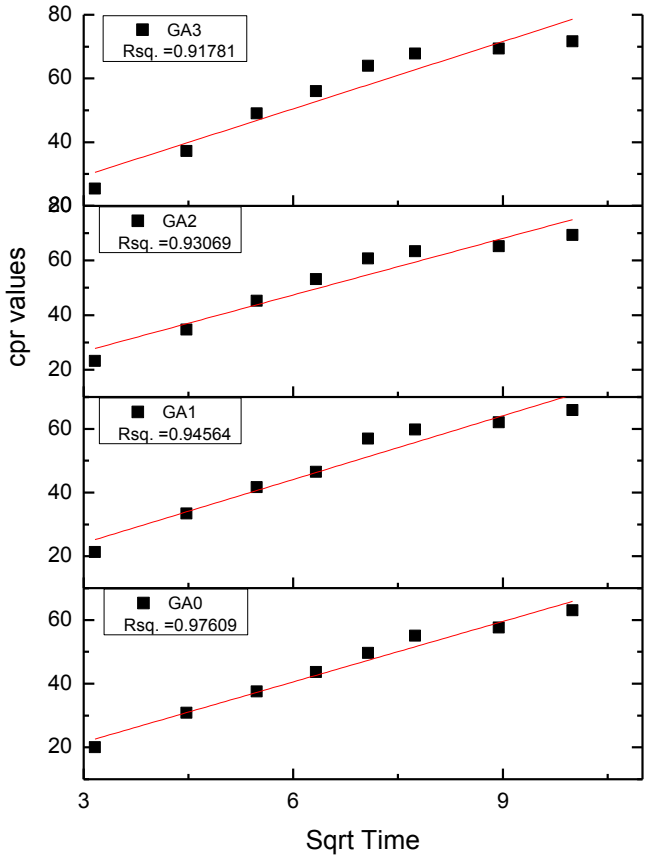


Figure 11.Graphical plot of percentage drug released vs. sqrt. time (Higuchi model), Gelatin-Alginate gels

Chapter 4

Conclusion

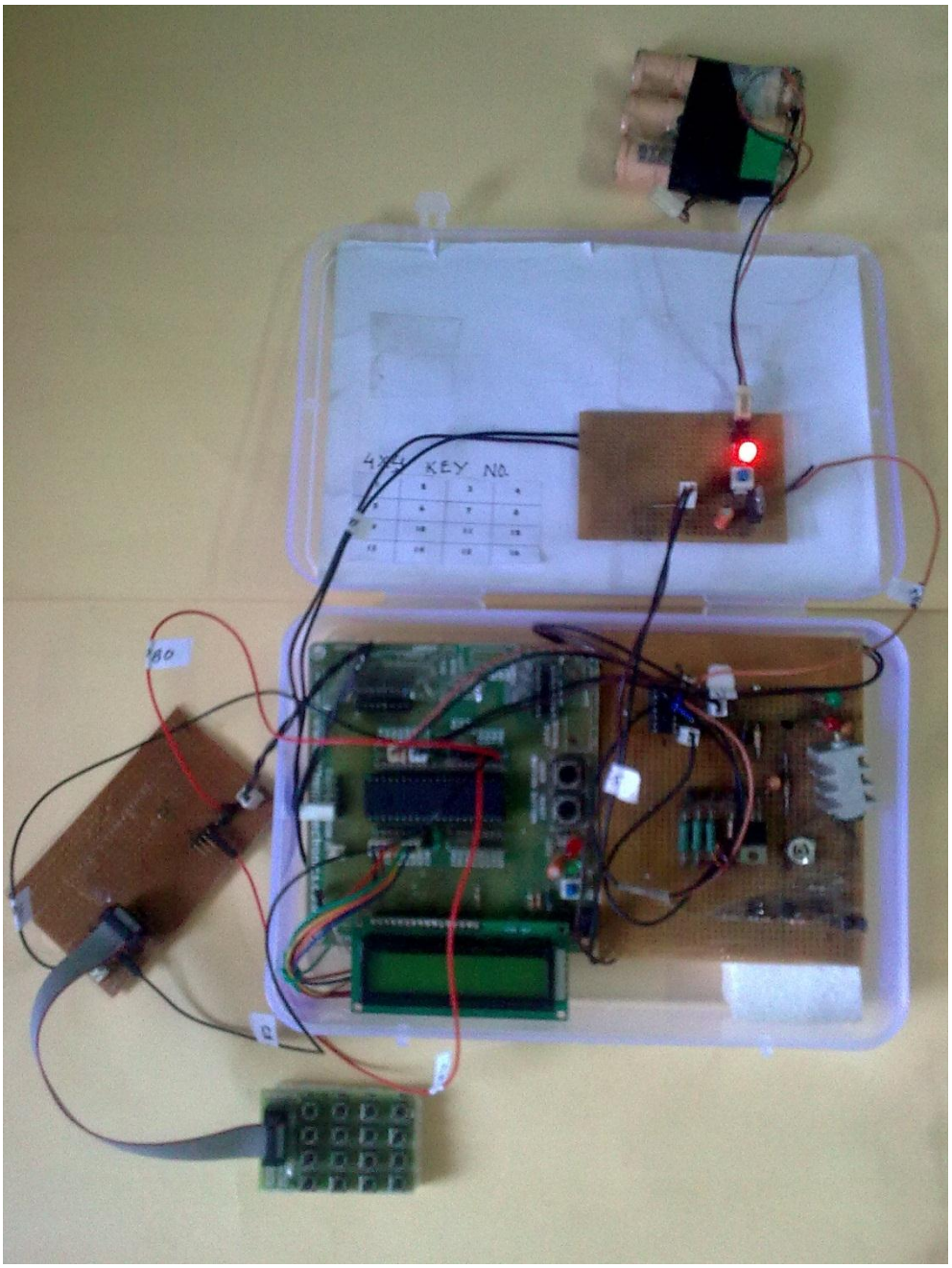
In the current study, successful attempts were made to develop low-cost, portable and user-friendly iontophoretic delivery system. The system was tested by using polymers, either alone or in combination with each other, as a matrix for Salicylic acid (model drug). The release study indicated that with increase in the duty cycle of the pulsed DC voltage there was a subsequent increase in the release rate. Salicylic acid (anionic) was hindered when Chitosan based gels were used; whereas when Alginate based gels were used there was an increase in release rate. Gelatin, being neutral in nature, showed release rate which were in between release rates of chitosan and Alginate based gels. All the matrix system showed Higuchian kinetics of release.

References

1. Kalia, Y.N., et al., *Iontophoretic drug delivery.* Advanced drug delivery reviews, 2004. **56**(5): p. 619-658.
2. Chien, Y.W., et al., *Direct current iontophoretic transdermal delivery of peptide and protein drugs.* Journal of pharmaceutical sciences, 1989. **78**(5): p. 376-383.
3. Pauwels, R., et al., *COPD exacerbations: the importance of a standard definition.* Respiratory medicine, 2004. **98**(2): p. 99-107.
4. Balant, L., E. Doelker, and P. Buri, *Prodrugs for the improvement of drug absorption via different routes of administration.* European journal of drug metabolism and pharmacokinetics, 1990. **15**(2): p. 143-153.
5. Dixit, N., et al., *Iontophoresis-an approach for controlled drug delivery: a review.* Current drug delivery, 2007. **4**(1): p. 1-10.
6. Nair, V., et al., *Transdermal iontophoresis. Part I: Basic principles and considerations.* Methods Find. Exp. Clin. Pharmacol, 1999. **21**(2).
7. Hirvonen, J., *Topical Iontophoretic Delivery: Progress to Date and Therapeutic Potential.* American Journal of Drug Delivery, 2005. **3**(2): p. 67-81.
8. Niamlang, S. and A. Sirivat, *Electric field assisted transdermal drug delivery from salicylic acid-loaded polyacrylamide hydrogels.* Drug Delivery, 2009. **16**(7): p. 378-388.
9. Schwartz, J.B., A.P. Simonelli, and W.I. Higuchi, *Drug release from wax matrices I. Analysis of data with first order kinetics and with the diffusion controlled model.* Journal of pharmaceutical sciences, 1968. **57**(2): p. 274-277.

Appendix

1. The following figure is the colour snap of the iontophoretic device



2. Driver program with LCD display, C-code

```
#include<avr/io.h>
#include"timer1.h"
#include"lcd.h"
#define hundred 15624
#define eighty 12500
#define sixty 9375
#define fifty 7812
#define fourty 6250
#define twenty 3125
void screen1(void);
void screen2(void);
void screen3(void);
void screen4(void);
void select(unsigned int duty);
void driver(char onoff);
unsigned int i=0;
int main()
{
	DDRB=0xff;
	timer_set_top(15624);
	timer_init(clk_64,Fast_pwm_top_ICR1,clear_on_cmp,'B');
	lcd_init();
	DDRD=0xFF;
	lcd_invisible_cursor();
	screen1();
	return 0;
}
void screen1()
{
	lcd_clear();
	lcd_position(0,0);
	lcd_print("IONTOPHORETIC DRUG DELIVERY");
	lcd_position(1,0);
	lcd_print("PRESS:1 TO CONTINUE");
	while(1)
	{
		lcd_scroll_left();
		if((PINB & 0xFF)== 0x01)
			break;
	}
	screen2();
	_delay_ms(30);
}
void screen2()
{
	lcd_clear();
	set_compare_register(0,'B');
	driver(0);
	lcd_position(0,0);
	lcd_print("Select Duty Cycle. 100%=1,80%=2");
	lcd_position(1,0);
	lcd_print("60%=3,50%=4,40%=5,20%=6,0%=7");
	while(1)
	{
		lcd_scroll_left();
```

```
                if((PINB & 0x0F)==1)
                {
                        i=hundred;
                        break;
                }
                if((PINB & 0x0F)==2)
                {
                        i=eighty;
                        break;
                }
                if((PINB & 0x0F)==4)
                {
                        i=sixty;
                        break;
                }
                if((PINB & 0x0F)==8)
                {
                        i=fifty;
                        break;
                }
                if((PINB & 0xFF)==0x10)
                {
                        i=fourty;
                        break;
                }
                if((PINB & 0xFF)==0x20)
                {
                        i=twenty;
                        break;
                }
                if((PINB & 0xFF)==0x40)
                {
                        i=0;
                        break;
                }
        }
        _delay_ms(30);
        screen3();
}

void screen3()
{
        lcd_clear();
        lcd_position(0,0);
        lcd_print("DUTY CYCLE SELECTED=");
        if(i==hundred)
                lcd_print(" 100%");
        if(i==eighty)
                lcd_print(" 80%");
        if(i==sixty)
                lcd_print(" 60%");
        if(i==fifty)
                lcd_print(" 50%");
        if(i==fourty)
                lcd_print(" 40%");
        if(i==twenty)
                lcd_print(" 20%");
```

```
	if(i==0)
		lcd_print(" 0%");
	lcd_position(1,0);
	lcd_print("PRESS:1 TO RUN, 2 TO RESELECT");
	while(1)
	{
		lcd_scroll_left();
		if((PINB & 0x0F)==1)
			screen4();
		if((PINB & 0x0F)==2)
			screen2();
	}
}
void select(unsigned int duty)
{
	set_compare_register(duty,'B');
	if(duty)
		driver(1);
	else
		driver(0);
}
void screen4()
{
	lcd_clear();
	if(i==hundred)
		lcd_print("Duty Cycle= 100%");
	if(i==eighty)
		lcd_print("Duty Cycle= 80%");
	if(i==sixty)
		lcd_print("Duty Cycle= 60%");
	if(i==fifty)
		lcd_print("Duty Cycle= 50%");
	if(i==fourty)
		lcd_print("Duty Cycle= 40%");
	if(i==twenty)
		lcd_print("Duty Cycle= 20%");
	if(i==0)
		lcd_print("Duty Cycle= 0%");
	select(i);
	lcd_position(1,0);
	lcd_print("RESELCT=1,PAUSE=2,RESUME=3");
	while(1)
	{
		lcd_scroll_left();
		if((PINB & 0xFF)== 0x01)
		{
			screen1();
			break;
		}
		if((PINB & 0xFF)== 0x02)
		{
			lcd_position(0,0);
			lcd_clear();
			lcd_print("PAUSED");
			lcd_position(1,0);
			lcd_print("RESELCT=1,PAUSE=2,RESUME=3");
			select(0);
```

```
                }
                if((PINB & 0xFF)== 0x04)
                {
                        lcd_clear();
                        if(i==hundred)
                                lcd_print("Duty Cycle= 100%");
                        if(i==eighty)
                                lcd_print("Duty Cycle= 80%");
                        if(i==sixty)
                                lcd_print("Duty Cycle= 60%");
                        if(i==fifty)
                                lcd_print("Duty Cycle= 50%");
                        if(i==fourty)
                                lcd_print("Duty Cycle= 40%");
                        if(i==twenty)
                                lcd_print("Duty Cycle= 20%");
                        if(i==0)
                                lcd_print("Duty Cycle= 0%");
                        select(i);
                        lcd_position(1,0);
                        lcd_print("RESELCT=1,PAUSE=2,RESUME=3");
                }
        }
}
void driver(char onoff)
{
        if(onoff==1)
                PORTD|=0x01;
        if(onoff==0)
                PORTD&=~(0x01);
}
```